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=> s reverse transcri##### and single stranded binding protein#
L1            9 REVERSE TRANSCRI##### AND SINGLE STRANDED BINDING PROTEIN#

=> s l1 and mRNA
L2            2 L1 AND MRNA

=> dup rem l2
PROCESSING COMPLETED FOR L2
L3            2 DUP REM L2 (0 DUPLICATES REMOVED)

=> d l3 1-2 bib ab kwic

L3   ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2003 ACS
AN   1997:568319  CAPLUS
DN   127:217138
TI   Determining exposure to ionizing radiation agent with persistent
     biological markers and identification of biological markers using
     differential display
IN   Goltry, Kristin L.; Greenberger, Joel S.
PA   University of Pittsburgh, USA
SO   PCT Int. Appl., 34 pp.
     CODEN: PIXXD2
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ---------------   ----  --------       ---------------  --------
PI   WO 9730179         A1   19970821       WO 1997-US1972   19970218
         W:  AU, CA, CN, JP, KR, MX
         RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
     US 6025336         A    20000215       US 1996-602145   19960215
     CA 2246387         AA   19970821       CA 1997-2246387  19970218
     AU 9722629         A1   19970902       AU 1997-22629    19970218
     EP 880605          A2   19981202       EP 1997-905832   19970218
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
             IE, FI
     CN 1216069         A    19990505       CN 1997-193746   19970218
     JP 2000504583      T2   20000418       JP 1997-529401   19970218
PRAI US 1996-602145          19960215
     WO 1997-US1972          19970218
AB   Persistent biol. indicators of exposure to ionizing radiation,
     particularly nucleic acid indicators, can be employed in detg. whether a
     subject has been exposed to ionizing radiation.  Such biol. indicators can
     be identified via the technique of differential display.  Differential
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display anal. of a mice cell line exposed to X-rays indicated that the expression of the serum amyloid A3 gene was increased while expression of the c-myc upstream **single-stranded binding proteins** MSSP-1 and MSSP-2 genes were decreased. In vivo exposure of mice to ionizing radiation induced SAA3 **mRNA** in bone marrow stromal cells. The increased **mRNA** levels was detectable for up to one year after exposure.

AB Persistent biol. indicators of exposure to ionizing radiation, particularly nucleic acid indicators, can be employed in detg. whether a subject has been exposed to ionizing radiation. Such biol. indicators can be identified via the technique of differential display. Differential display anal. of a mice cell line exposed to X-rays indicated that the expression of the serum amyloid A3 gene was increased while expression of the c-myc upstream **single-stranded binding proteins** MSSP-1 and MSSP-2 genes were decreased. In vivo exposure of mice to ionizing radiation induced SAA3 **mRNA** in bone marrow stromal cells. The increased **mRNA** levels was detectable for up to one year after exposure.

IT Proteins, specific or class
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(MSSP-1 (c-myc upstream **single-stranded binding protein** 1); detg. exposure to ionizing radiation agent with persistent biol. markers and identification of biol. markers using differential display)

IT Proteins, specific or class
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(MSSP-2 (c-myc upstream **single-stranded binding protein** 1); detg. exposure to ionizing radiation agent with persistent biol. markers and identification of biol. markers using differential display)

IT PCR (polymerase chain reaction)
(**reverse transcription**; detg. exposure to ionizing radiation agent with persistent biol. markers and identification of biol. markers using differential display)

L3 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS
AN 1993:1623 CAPLUS
DN 118:1623
TI Direct PCR sequencing of murine immunoglobulin genes using E. coli single-stranded DNA-binding protein
AU Rapley, Ralph; Flora, Singh; Walker, Matthew R.
CS Med. Sch., Birmingham Univ., Birmingham, B15 2TH, UK
SO PCR Methods and Applications (1992), 2(1), 99-101
CODEN: PMAPES; ISSN: 1054-9803
DT Journal
LA English

AB The authors investigated the use of Escherichia coli **single-stranded binding protein** in an attempt to prevent the rapid reannealing of PCR strands following duplex denaturation, and to allow uninterrupted extension and specific termination over the increased lengths of the PCR fragment. A no. of murine hybridoma cell lines secreting IgG1 monoclonal antibodies to a synthetic peptide representing the 1-34 amino acid region of parathyroid hormone-related protein (PTHrP) were used in this study. Initially the **mRNA** encoding the heavy-chain variable region from one of these hybridomas (cell line E2) was isolated and subjected to first-strand cDNA synthesis with **reverse transcriptase**. PCR was then carried out using a degenerate reverse primer D750 and a forward primer D751. These primers annealed to heavy-chain framework region 1 and const. region 1 (26 bp from the D-J junction), resp., and amplified a predominant 400-bp fragment.

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denaturation, and to allow uninterrupted extension and specific termination over the increased lengths of the PCR fragment. A no. of murine hybridoma cell lines secreting IgG1 monoclonal antibodies to a synthetic peptide representing the 1-34 amino acid region of parathyroid hormone-related protein (PTHrP) were used in this study. Initially the **mRNA** encoding the heavy-chain variable region from one of these hybridomas (cell line E2) was isolated and subjected to first-strand cDNA synthesis with **reverse transcriptase**. PCR was then carried out using a degenerate reverse primer D750 and a forward primer D751. These primers annealed to heavy-chain framework region 1 and const. region 1 (26 bp from the D-J junction), resp., and amplified a predominant 400-bp fragment.

```
=> s l2 and length
L4            0 L2 AND LENGTH

=> s l1 and (length(10a)mRNA)
L5            0 L1 AND (LENGTH(10A) MRNA)

=> s l1 and length
L6            4 L1 AND LENGTH

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7            1 DUP REM L6 (3 DUPLICATES REMOVED)

=> d l7 bib ab kwic

L7   ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2003 ACS         DUPLICATE 1
AN   1996:75965  CAPLUS
DN   124:137651
TI   Strand displacement synthesis of the long terminal repeats by HIV
     reverse transcriptase
AU   Fuentes, Gloria M.; Rodriguez-Rodriguez, Lorna; Palaniappan, Chockalingam;
     Fay, Philip J.; Bambara, Robert A.
CS   Departments of Microbiology Immunology, Univ. of Rochester, Rochester, NY,
     14642, USA
SO   Journal of Biological Chemistry (1996), 271(4), 1966-71
     CODEN: JBCHA3; ISSN: 0021-9258
PB   American Society for Biochemistry and Molecular Biology
DT   Journal
LA   English
AB   According to the current model for retroviral replication, strand
     displacement of the long terminal repeat (LTR) is a necessary step during
     plus strand DNA synthesis in vivo. The ability of human immunodeficiency
     virus reverse transcriptase (HIV-RT) to synthesize in
     vitro over a 634-nucleotide HIV LTR DNA template, having or lacking a
     single full-length DNA downstream primer, was investigated. The
     presence of the down-stream primer resulted in an .apprx.12-fold redn. in
     the rate of upstream primer elongation. Addn. of Escherichia coli
     single-stranded binding protein
     (SSB) or human replication protein A (RP-A) enhanced strand displacement
     synthesis; however, addn. of HIV nucleocapsid protein (NC) did not. The
     presence of excess single-stranded DNA complementary to the downstream
     primer did not stimulate displacement synthesis. Interestingly, the
     elongating upstream primer could readily transfer to this DNA. This
     observation suggests that recombination is favored during strand
     displacement synthesis in vivo.
TI   Strand displacement synthesis of the long terminal repeats by HIV
     reverse transcriptase
AB   According to the current model for retroviral replication, strand
     displacement of the long terminal repeat (LTR) is a necessary step during
     plus strand DNA synthesis in vivo. The ability of human immunodeficiency
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virus **reverse transcriptase** (HIV-RT) to synthesize in vitro over a 634-nucleotide HIV LTR DNA template, having or lacking a single full-**length** DNA downstream primer, was investigated. The presence of the down-stream primer resulted in an .apprx.12-fold redn. in the rate of upstream primer elongation. Addn. of Escherichia coli **single-stranded binding protein** (SSB) or human replication protein A (RP-A) enhanced strand displacement synthesis; however, addn. of HIV nucleocapsid protein (NC) did not. The presence of excess single-stranded DNA complementary to the downstream primer did not stimulate displacement synthesis. Interestingly, the elongating upstream primer could readily transfer to this DNA. This observation suggests that recombination is favored during strand displacement synthesis in vivo.

ST LTR strand displacement HIV virus; **reverse transcription** strand displacement HIV virus

IT **Reverse transcription**
(strand displacement synthesis of the long terminal repeats by HIV **reverse transcriptase**)

IT Deoxyribonucleic acid formation factors
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(RF-A (replication factor A), strand displacement synthesis of the long terminal repeats by HIV **reverse transcriptase**)

IT Proteins, specific or class
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(SSB (single-stranded DNA-binding), strand displacement synthesis of the long terminal repeats by HIV **reverse transcriptase**)

IT Virus, animal
(human immunodeficiency 1, strand displacement synthesis of the long terminal repeats by HIV **reverse transcriptase**)

IT Genetic element
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(long terminal repeat, strand displacement synthesis of the long terminal repeats by HIV **reverse transcriptase**)

IT 9068-38-6, **Reverse transcriptase**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(strand displacement synthesis of the long terminal repeats by HIV **reverse transcriptase**)

=>

10038.177

WEST

Freeform Search

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Term: L4 and concentration

Display: 10 **Documents in Display Format:** - **Starting with Number** 1

Generate: ○ Hit List ◎ Hit Count ○ Side by Side ○ Image

Search | Clear | Help | Logout | Interrupt

Main Menu | Show S Numbers | Edit S Numbers | Preferences | Cases

Search History

DATE: Wednesday, July 16, 2003 Printable Copy Create Case

Set Name (side by side)	Query	Hit Count	Set Name (result set)
DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L5	L4 and concentration	7	L5
L4	L3 and (mRNA near5 length)	8	L4
L3	reverse transcri$5 and single stranded binding protein$1	51	L3
L2	amplif$7 and single binid	0	L2
DB=DWPI,USPT,EPAB,JPAB; PLUR=YES; OP=ADJ			
L1	reverse transcri$5 and single binding protein$1	0	L1

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 7 of 7 returned.

☐ 1. 6573060 . 28 Nov 00; 03 Jun 03. Methods and compositions for targeting DNA metabolic processes using aminoglycoside derivatives. Hockensmith; Joel W., et al. 435/15; 435/18 536/13.2 536/13.3 536/13.6 536/13.7 536/4.1. C12Q001/48 C12Q001/34 C07H015/22 C07H015/232 C07H015/234.

☐ 2. 6537791 . 28 Nov 00; 25 Mar 03. Mammalian DNA-dependent ATPase a polypeptides and fusions thereof. Hockensmith; Joel W., et al. 435/196; 435/195 536/23.2 536/23.4. C12N009/16 C12N009/14 C07H021/04.

☐ 3. 6531306 . 28 Nov 00; 11 Mar 03. Polynucleotides encoding mammalian DNA-dependent ATPase A polypeptides. Hockensmith; Joel W., et al. 435/196; 435/252.3 435/254.4 435/320.1 435/325 435/366 536/23.2. C12N009/16 C12N001/21 C12N005/10 C12N015/52 C07H021/04.

☑ 4. 6495320 . 21 Jul 99; 17 Dec 02. Even length proportional amplification of nucleic acids. Lockhart; David J., et al. 435/6; 435/91.2 435/91.51 536/24.3. C12Q001/68 C12P019/34 C07H021/04.

☐ 5. 6238905 . 12 Sep 97; 29 May 01. Thermophilic polymerase III holoenzyme. McHenry; Charles S., et al. 435/252.3; 435/194 435/254.11 435/320.1 435/325 435/419 530/326 536/23.2 536/23.7. C12N009/12 C12N001/20 C12N015/00 C07H021/04.

☐ 6. 6180612 . 27 Oct 98; 30 Jan 01. Methods and compositions for targeting DNA metabolic processes using aminoglycoside derivatives. Hockensmith; Joel W., et al. 514/25; 514/39 514/41. A01N043/04.

☐ 7. 5089396 . 05 Jul 88; 18 Feb 92. Nucleic acid encoding .beta. chain prodomains of inhibin and method for synthesizing polypeptides using such nucleic acid. Mason; Anthony J., et al. 435/69.1; 435/252.3 435/320.1 435/360 435/69.4 536/23.51. C12P021/02 C12N015/11 C12N001/21 C12N005/10.

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Term	Documents
CONCENTRATION	745305
CONCN	157552
CONCNS	18248
CONCENTRATIONS	273732
(4 AND CONCENTRATION).USPT,JPAB,EPAB,DWPI.	7
(L4 AND CONCENTRATION).USPT,JPAB,EPAB,DWPI.	7

Previous Page Next Page

☐ Generate Collection

L5: Entry 5 of 7 File: USPT May 29, 2001

DOCUMENT-IDENTIFIER: US 6238905 B1
TITLE: Thermophilic polymerase III holoenzyme

Brief Summary Text (24):
The present invention also provides methods for detecting DNA polymerase III holoenzyme or holoenzyme subunit expression comprising the steps of: a) providing a sample suspected of containing DNA polymerase III holoenzyme or holoenzyme III subunit; and a control containing a quantitated DNA polymerase III holoenzyme or holoenzyme III subunit protein, as appropriate; and b) comparing the test DNA polymerase III holoenzyme or holoenzyme subunit, in the sample with the quantitated DNA polymerase III holoenzyme or holoenzyme subunit in the control to determine the relative concentration of the test DNA polymerase III holoenzyme or holoenzyme III subunit in the sample. In addition, the methods may be conducted using any suitable means to determine the relative concentration of DNA polymerase III holoenzyme or holoenzyme subunit in the test and control samples, including but not limited to the means selected from the group consisting of Western blot analysis, Northern blot analysis, Southern blot analysis, denaturing polyacrylamide gel electrophoresis, reverse transcriptase-coupled polymerase chain reaction, enzyme-linked immunosorbent assay, radioimmunoassay, and fluorescent immunoassay. Thus, the methods may be conducted to determine the presence of DNA polymerase III holoenzyme or holoenzyme III subunit in the genome of the source of the test sample, or the expression of DNA polymerase III holoenzyme or holoenzyme subunit (mRNA or protein), as well as detect the presence of abnormal or mutated DNA polymerase holoenzyme or holoenzyme subunit proteins or gene sequences in the test samples.

Detailed Description Text (7):
The term "gene" refers to a nucleic acid (e.g., DNA) sequence that comprises coding sequences necessary for the production of a polypeptide or precursor (e.g., DNA polymerase III holoenzyme or holoenzyme subunit, as appropriate). The polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence so long as the desired activity or functional properties (e.g., enzymatic activity, ligand binding, signal transduction, etc.) of the full-length or fragment are retained. The term also encompasses the coding region of a structural gene and the including sequences located adjacent to the coding region on both the 5' and 3' ends for a distance of about 1 kb on either end such that the gene corresponds to the length of the full-length mRNA. The term "gene" encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region interrupted with non-coding sequences termed "intervening regions" or "intervening sequences." The MRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide.

Detailed Description Text (13):
The term "oligonucleotide" as used herein is defined as a molecule comprised of two or more deoxyribonucleotides or ribonucleotides, preferably more than three, and usually more than ten. The exact size will depend on many factors, which in turn depends on the ultimate function or use of the oligonucleotide. The oligonucleotide may be generated in any manner, including chemical synthesis, DNA replication, reverse transcription, or a combination thereof.

Detailed Description Text (28):
"Hybridization" methods involve the annealing of a complementary sequence to the target nucleic acid (the sequence to be detected). The ability of two polymers of nucleic acid containing complementary sequences to find each other and anneal through base pairing interaction is a well-recognized phenomenon. The initial observations of the "hybridization" process by Marmur and Lane, (See e.g., Marmur and Lane, Proc. Natl. Acad. Sci. USA 46:453 [1960]); and Doty et al., Proc. Natl. Acad. Sci. USA

46:461 [1960]) have been followed by the refinement of this process into an essential tool of modem biology. Nonetheless, a number of problems have prevented the wide scale use of hybridization as a tool in diagnostics. Among the more formidable problems are: 1) the inefficiency of hybridization; 2) the low concentration of specific target sequences in a mixture of genomic DNA; and 3) the hybridization of only partially complementary probes and targets.

Detailed Description Text (31):
Solution hybridization of oligonucleotide probes to denatured double-stranded DNA is further complicated by the fact that the longer complementary target strands can renature or reanneal. Again, hybridized probe is displaced by this process. This results in a low-yield of hybridization (low "coverage") relative to the starting concentrations of probe and target.

Detailed Description Text (32):
With regard to low target sequence concentration, the DNA fragment containing the target sequence is usually in relatively low abundance in genomic DNA. This presents great technical difficulties; most conventional methods that use oligonucleotide probes lack the sensitivity necessary to detect hybridization at such low levels.

Detailed Description Text (33):
One attempt at a solution to the target sequence concentration problem is the amplification of the detection signal. Most often this entails placing one or more labels on an oligonucleotide probe. In the case of non-radioactive labels, even the highest affinity reagents have been found to be unsuitable for the detection of single copy genes in genomic DNA with oligonucleotide probes. (See, Wallace et al., Biochimie 67:755 [1985]). In the case of radioactive oligonucleotide probes, only extremely high specific activities are found to show satisfactory results. (See, Studencki and Wallace, DNA 3:1 [1984] and Studencki et al., Human Genetics 37:42 [1985]).

Detailed Description Text (38):
The art knows well that numerous equivalent conditions may be employed to comprise low stringency conditions; factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA, RNA, base composition, present in solution or immobilized, etc.) and the concentration of the salts and other components (e.g., the presence or absence of formamide, dextran sulfate, polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of low stringency hybridization different from, but equivalent to, the above listed conditions. In addition, the art knows conditions which promote hybridization under conditions of high stringency (e.g., increasing the temperature of the hybridization and/or wash steps, the use of formamide in the hybridization solution, etc.).

Detailed Description Text (56):
As used herein, the term "polymerase chain reaction" ("PCR") refers to the method of K. B. Mullis U.S. Pat. Nos. 4,683,195 4,683,202, and 4,965,188, hereby incorporated by reference, which describe a method for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. This process for amplifying the target sequence consists of introducing a large excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence, followed by a precise sequence of thermal cycling in the presence of a DNA polymerase. The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, the mixture is denatured and the primers then annealed to their complementary sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing and polymerase extension can be repeated many times (i e., denaturation, annealing and extension constitute one "cycle"; there can be numerous "cycles") to obtain a high concentration of an amplified segment of the desired target sequence. The length of the amplified segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the "polymerase chain reaction" (hereinafter "PCR"). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified".

Detailed Description Text (61):
As used herein, the term "RT-PCR" refers to the replication and amplification of RNA sequences. In this method, reverse transcription is coupled to PCR, most often using a one enzyme procedure in which a thermostable polymerase is employed, as described in U.S. Pat. No. 5,322,770, herein incorporated by reference. In RT-PCR, the RNA template is converted to cDNA due to the reverse transcriptase activity of the polymerase, and then amplified using the polymerizing activity of the polymerase (ie., as in other PCR methods).

Detailed Description Text (89):
The terms "test DNA polymerase III holoenzyme" and "test holoenzyme subunit" refers to a sample suspected of containing DNA polymerase III holoenzyme or holoenzyme subunit, respectively. The concentration of DNA polymerase III holoenzyme or holoenzyme subunit in the test sample is determined by various means, and may be compared with a "quantitated amount of DNA polymerase III holoenzyme or holoenzyme subunit" (i.e., a positive control sample containing a known amount of DNA polymerase III holoenzyme or holoenzyme subunit), in order to determine whether the concentration of test DNA polymerase III holoenzyme or holoenzyme subunit in the sample is within the range usually found within samples from wild-type organisms.

Detailed Description Text (104):
In the experimental disclosure which follows, the following abbreviations apply: g (gram); L (liter); .mu.g (microgram); ml (milliliter); bp (base pair); .degree. C. (degrees Centigrade); kb or Kb (kilobases); kDa or kd (kilodaltons); EDTA (ethylenediaminetetraacetic acid); DTT (dithiothreitol); LB (Luria Broth); -mer (oligomer); DMV (DMV International, Frazier, NY); PAGE (polyacrylamide gel electrophoresis); SDS (sodium dodecyl sulfate); SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis); SSPE (2.times.SSPE contains 0.36 mM NaCl, 20 mM NaH.sub.2 PO.sub.4, pH 7.4, and 20 mM EDTA, pH 7.4; the concentration of SSPE used may vary), SOP media (20 g/l tryptone (Difco), 10 g/l yeast extract (Difco), 5 g/l NaCl, 2.5 g/l potassium phosphate, dibasic (Fisher), 1 g/l MgSO.sub.4.7H.sub.2 O (Fisher), pH 7.2); TE buffer (10 mM Tris, 1 mM EDTA); 50.times.TAE (242 g Tris base, 57.1 ml glacial acetic acid, 100 ml 0.5 M EDTA pH 8.0); Blotto (10% skim milk dissolved in dH.sub.2 O and 0.2% sodium azide); Gel Loading Dye (0.25% Bromophenol blue, 0.25% xylene cyanol, 25% Ficoll (Type 400) in dH.sub.2 O); Pre-hybridization mix (50% Formamide, 5.times.SSPE, 1% SDS, 0.5% CARNATION1 non-fat dried milk, 10% skim milk, 0.2% Na Azide); FBS (fetal bovine serum); ABS, Inc. (ABS, Inc., Wilmington, Del.); Boehringer Mannheim (Boehringer Mannheim, Indianapolis, Ind.); Champion Industries (Champion Industries, Clifton, N.J.); Organon (Organon Teknika Corp., Durham N.C.); Difco (Difco, Detroit, Mich.); Enzyco (Enzyco Inc., Denver, Colo.); Fisher Scientific (Fisher Scientific, Fair Lawn, N.J.); FMC (FMC, Rockland, Me.); Gibco BRL (Gibco BRL Gaithersburg, Md.); Hyclone (Hyclone, Logan Utah); Intermountain or ISC (ISC BioExpress, Bountiful, Utah); Invitrogen (Invitrogen, Carlsbad, Calif.); Millipore (Millipore, Marlborough, Mass.); MJ Research (MJ Research, Watertown, Mass.); Molecular Probes (Molecular Probes, Eugene, Oreg.); National Diagnostics (National Diagnostics, Manville, N.J.); Pharmacia Biotech (Pharmacia Biotech., Piscataway, N.J.); Promega (Promega Corp., Madison, Wis.); Sigma (Sigma, St. Louis, Mo.); Stratagene (Stratagene, LaJolla Calif.); Tecan (Tecan, Research Triangle Park, N.C.); Whatman (Whatman, Maidstone, England); Lofstrand Labs (Lofstrand Labs, Ltd., Gaithersburg, Md.) and LSPI (LSPI Filtration Products, Life Science Products, Denver, Col.); Irvine (Irvine Scientific, Irvine Calif.); and Jackson Labs (Jackson Labs, Bar Harbor, Me.).

Detailed Description Text (110):
The M13Gori template (Enzyco) was first primed with RNA as described below. The following components were mixed together on ice in the following order: 243 li of primer-template solution (60 mM HEPES (pH 7.5), 14 mM magnesium acetate, 2.8 mM ATP, GTP, CTP and UTP, 14% glycerol, 56 mM NaCl, 42 mM potassium glutamate, 84 .mu.g/ml bovine serum albumin (BSA) and 4 mM DTT), with 7.2 .mu.l M13Gori (2.98 mg/ml), 63 .mu.l E. coli single-stranded binding protein (SSB) (Enzyco) (2.2 mg/ml) and 27 .mu.l DnaG primase (Enzyco) (0.39 mg/ml). This mixture was then incubated for 15 min at 30.degree. C. to form the "primer-template." The above mixture provided enough primed M13Gori for over 100 polymerase assays.

Detailed Description Text (150):
For cloning by the limiting dilution technique, cells were removed from the chosen positive wells and placed in a 15 ml conical tube. An aliquot of 600,000 cells was taken and added to a tube containing RPMI-serum free media to bring the final cell density to 60,000 cells/ml. Cells were diluted 1:10 successively with RPM-serum free media until a dilution of 600 cells/nl was achieved. Cells were then diluted with HCS (Hybridoma Cloning Supplement, Boehringer Mannheim) until concentrations of 50, 10 and 5 cells/ml were achieved. These were then aliquoted into wells of a 96 well plate (2 drops/well), incubated in a 37.degree. C. incubator and visually scored after five days in an inverted microscope, by noting the wells that appear to have colonies that arose from a single cell (one colony/well). These wells were screened by ELISA to identify clones that are producing antibody directed against .alpha..

Detailed Description Text (190):
Thermus thermophilus strain pMAF.kat (obtained from J. Berenguer; See, Lase et al, [1992]) genomic DNA was prepared using previously described methods (Ausubel FM et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York NY [1995]). Briefly, T. thermophilus strain pMAF.kat was grown overnight at 70.degree. C. from a single colony in 100 ml of Thermus rich media (8 g/l Bacto-tryptone (Difco), 4 g/l yeast extract (Difco), 3 g/l NaCl (Fisher Scientific) (pH 7.5) with vigorous shaking in a New Brunswick Model G25 incubator. The culture was centrifuged in a 250 ml centrifuge bottle in a Sorvall GSA rotor at 6000 rpm (5858.times.g) for 6 minutes, followed by resuspension of the pellet from a 100 ml overnight culture in 9.5 ml TE buffer (pH 7.5) in an SS34 centrifuge tube (Sorvall). 0.5 ml 10% SDS and 50 .mu.l of fungal proteinase K (20 mg/ml in dH.sub.2 0) (GibcoBRL) was mixed with the suspension, the mixture was incubated at 37.degree. C. for 1 h, followed by addition of 2.35 ml of 4M NaCl (Fisher Scientific), and 1.6 ml of cetyltrimethylammonium bromide (CTAB)/NaCl solution (10% CTAB in 0.7 M NaCl). The resulting mixture was incubated at 65.degree. C. for 20 minutes followed by extraction with an equal volume of chloroform/isoamyl alcohol (30:1 mixture). The mixture was centrifuged in an SS34 rotor (Sorvall) for 10 minutes at 6000.times.g, 0.6 volumes of isopropanol (Fisher) was added to the pellet to form a stringy, white precipitate. The stringy white precipitate was placed into 25 ml 75% ethanol, centrifuged for 5 minutes at 10,000.times.g and the supernatant discarded. The pellet was resuspended in 4 ml of TE buffer (pH 7.5), the DNA concentration measured by determining the absorbance at a wavelength of 260 nm in a spectrophotometer, and the DNA concentration adjusted to 100 .mu.g/ml with TE (pH 7.5). 4.3 g of cesium chloride (CsCl) (Sigma cat. # C-4036) and 200 .mu.l of ethidium bromide (10 mg/ml) were added per 4 ml of TE (pH 7.5) used to resuspend the DNA pellet. The mixture was centrifuged in a Sorvall T-1270 rotor in a Sorvall RC70B ultracentrifuge at 55,000 rpm, at 15.degree. C. overnight. The chromosomal DNA band (highlighted with UV light) was removed, the ethidium bromide extracted by adding an equal volume of H.sub.2 O saturated butanol, mixing thoroughly by inverting the tube, and briefly centrifuging the tube (30 seconds at 14,000 g) to separate the phases. The butanol extraction was repeated until pink color could not be detected, and then the extraction was repeated one more time. The remaining DNA/cesium chloride/TE (pH 7.5) mixture was dialyzed overnight in 500.times.volume of TE (pH 7.5) to remove the cesium chloride. DNA was precipitated by the addition of 1/10 volume of 2.5 M sodium acetate (pH adjusted to 5.2 with glacial acetic acid) and 1 volume of isopropanol, and centrifugation at 11,951.times.g for 10 minutes. The pellet was washed with 75% ethanol and centrifuged at 11,951.times.g for 2 minutes. The supernatant was removed, and the wash repeated. After the final wash, the supernatant was removed and the pellet allowed to dry until it became slightly translucent. The pellet was resuspended in 25 ml of TE (pH 7.5) by gentle rocking overnight at room temperature. DNA was quantitated by spectrophotometry by taking a spectrum of wavelengths from 220 nm to 340 nm. Two preparations were obtained. Preparation A diluted 1/70 in TE buffer gave an OD.sub.260 of 0.175 consistent with a DNA concentration of 0.612 mg/ml using as a conversion factor A.sub.260 =1=50 .mu.g/ml. Preparation B, with a DNA concentration of 0.906.

Detailed Description Text (208):
PCR-amplified sequences were extracted from gel slices prepared as described above using freeze-squeeze extraction. Extraction was performed by placing the gel slice in a sterile micro-spin device (0.45 mm pore size cellulose acetate filter (LSPI) that was then inserted into a 2.0 ml Eppendorf tube and the assembly was placed in a -80.degree. C. freezer for 10 minutes. The following steps were performed at room

temperature. The frozen gel slice was then centrifuged in the micro-spin assembly for 4 minutes at maximum rpm in a microcentrifuge (14,000.times.g). The crushed gel slice was then resuspended in 100 .mu.l of 2.5 M Na Acetate, re-frozen for 10 minutes at -80.degree. C., and centrifuged as before. The liquid containing the gel slice DNA was collected in the bottom of the Eppendorf tube (approximately 300 .mu.l) and the DNA was precipitated by the addition of 1 .mu.l glycogen (Boehringer Mannheim) and an equal volume (300 .mu.l) of isopropanol. The precipitate mixture was centrifuged (14,000.times.g) in a microcentrifuge for 10 minutes, the supernatant discarded, and the pellet washed with 75% ethanol. The ethanol wash supernatant was discarded, and the pellet was resuspended in 5 .mu.l TE (pH 7.5). The DNA was quantitated by adding 1 .mu.l of DNA to 300 .mu.l of a 1/400 dilution in TE (pH 7.5) of PicoGreen.TM. (Molecular Probes) in a well of a 96-well microtiter plate (Life Sciences), and compared to known concentrations of Thermus thermophilus genomic DNA by excitation on an SLT Fluostar Microplate Fluorometer of the PicoGreen.TM. at 480 nm and measuring emission at 520 nm.

Detailed Description Text (222):
Probe DNA was prepared from vectors (pCRII, Invitrogen) containing genomic DNA segments which encode portions of subunits of the Thermus thermophilus DNA polymerase III holoenzyme subunits, as described above. The gene segments were released from the vector by digestion of 10 .mu.g of the cloned DNA's in a 300 .mu.l reaction with EcoRI (100 Units, Gibco BRL) in 1.times. Boehringer Mannheim High Salt restriction digest buffer H at 37.degree. C. for 2 hours 60 II of gel loading dye was added to each digest, the entire 360 .mu.l digest was loaded into 260.times.1.5 mm wells, and the digests were electrophoresed in 4% NuSieve GTG agarose (FMC) in 1.times.TAE buffer, and the gel was run at 100 V (constant voltage) for 4 hours. The gel was stained with 300 ml Sybr green (Molecular Probes, diluted 1/10,000 in 1.times.TAE) for 1 h with gentle rocking. Bands containing the gene fragments were excised with a clean scalpel, placed into a tared 4 ml Nunc tube (Nunc 4.5 ml Cryotube, NUNC), and the bands were weighed. The slices were melted by putting the tubes in a gravity convection incubator at 72.degree. C. for 30 minutes or until the slice was completely melted. Once melted the tubes were cooled to 45.degree. C. in a temperature block and 1/10 volume of 10.times. .beta.-agarase buffer (Gibco BRL; 100 mM Bis-Tris (pH 6.5), 10 mM EDTA) was added, followed by the addition of 4 .mu.l of .beta.-Agarase (GibcoBRL, 1 unit/.mu.l). The mixture was incubated overnight in a gravity convection incubator at 42.degree. C. To precipitate the DNA, 1/10 vol. of 3 M NaOAc (pH 5.2), was added and the mixture chilled on ice. Any remaining undigested agarose was removed by centrifugation for 2 minutes in 2 ml Eppendorf tubes at 14,000.times.g in an Eppendorf microcentrifuge. The supernatant was removed and the DNA precipitated by the addition of 1 ml glycogen (Boehringer Mannheim, molecular biology grade, 20 mg/ml) and 2.5 volumes of ethanol, followed by centrifugation at 14,000.times.g in an Eppendorf microcentrifuge for 10 minutes. The supernatant was removed and the pellet washed with 0.5 ml of 75% ethanol and centrifuged at 14,000.times.g in an Eppendorf microcentrifuge for 2 minutes. The pellets were resuspended in 10 .mu.l of a 1/10 dilution of TE (1 mM Tris (pH 7.5), 0.1 mM EDTA final concentration). The DNA was quantitated by adding 1 .mu.l of DNA to 300 PI of a 1/400 dilution of PicoGreen.TM. in a well of a 96-well microtiter plate (Life Sciences), and compared to known concentrations of Thermus thermophilus genomic DNA by excitation of the PicoGreen.TM. at 480 nm and measuring emission at 520 nm, on an SLT Fluostar Microplate Fluorometer.

Detailed Description Text (224):
The probe was labeled by adding 50 ng of DNA to a random hexamer priming reaction mixture (Random Primed DNA Labeling Kit, Boehringer Mannheim) following the manufacture's instructions. Briefly, 50 ng DNA in 10 Pi dH.sub.2 O was placed into a 0.2 ml thin-walled PCR tube, overlaid with 25 pi mineral oil and the DNA denatured by heating at 95.degree. C. for 10 minutes in a thermal cycler (MJ Research). At 9 minutes through the 10 minute denaturation, 2 .mu.l of Boehringer Mannheim hexanucleotide reaction mix (tube 6 in kit) was added and the denaturation continued for an additional minute. Just prior to the end of the 10 minute denaturation, the mixture was rapidly cooled by removing the tube from the thermal cycler and immediately placing it in a beaker of ice-water. One .mu.l each of dATP, dTTP, dGTP (0.5 mM in Tris buffer, final concentration 25 FM) was added to the reaction, followed by 5 .mu.l of .sup..alpha.32 P-dCTP (50 .mu.Ci, 3000 Ci/mmole, in 5 mM Tris-HCl (pH 7.5)), and 1 .mu.l of Klenow DNA polymerase (Boehringer Mannheim, 2 units/el in 50% glycerol), and the mixture was incubated at 37.degree. C. in a water bath for 1 h.

Detailed Description Text (256):
The plasmid preparation was purified using a vacuum manifold by using ProMega Plus Minipreps (Promega, A721C) which can be easily processed simultaneously with Promega's Vac-Man.TM. or Vac-Man.TM. Jr. Laboratory Vacuum Manifold. One ProMega Plus Minipreps column was prepared for each miniprep. The ProMega Plus Minipreps resin (Promega, A767C) was thoroughly mixed before removing an aliquot. If crystals or aggregates were present, they were dissolved by warming the resin to 25-37.degree. C. for 10 minutes, then cooling to 30.degree. C. before use. One ml of the resuspended resin was pipetted into each barrel of the Minicolumn/Syringe assembly (i.e., the assembly formed by attaching the Syringe Barrels to the Luer-Lok.RTM. extension of each Minicolumn), and all of the cleared lysate from each plasmid preparation was transferred to the barrel of the Minicolumn/Syringe assembly containing the resin. A vacuum was applied to pull the resin/lysate mix into the Minicolumn until all of the sample has completely passed though the column. Extended incubation of the resin and lysate was not necessary since at the concentration of plasmid present in most lysates, plasmid binding to the resin was immediate. 320 ml of 95% ethanol was added to the Column Wash solution bottle to yield a Column Wash Solution (Promega, A810E) having a final concentration of 80 mM Potassium acetate, 8.3 mM Tris-HCl (pH 7.5), 40CM EDTA and 55% ethanol. 2 ml of the Column Wash Solution was added to the Syringe Barrel and the vacuum reapplied to draw the solution though the Minicolumn. The resin was dried by continuing to draw a vacuum for a maximum of 30 seconds after the solution has been pulled though the column. The Minicolumn was transferred to a 1.5 ml microcentrifuge tube and the Minicolumn centrifuged at 10,000.times.g in a microcentrifuge for 2 minutes to remove any residual Column Wash Solution. The Minicolumn was transferred to a new microcentrifuge tube, and eluted with 50 .mu.l water or TE buffer (elution in TE buffer was not used if the DNA was subsequently used in an enzyme reaction, particularly if the DNA was dilute and if large volumes of the DNA solution were required to be added to a reaction, since EDTA may inhibit some enzymes by chelating magnesium required as a co-factor for activity). DNA was eluted by centrifuging the tube at 10,000.times.g in a microcentrifuge for 20 seconds. For large plasmids (.gtoreq.10 kb), water or TE buffer preheated to 65-70.degree. C. was used since it may increase yields. For plasmids >20kb, water or TE buffer preheated to 80.degree. C. was used. Plasmids were eluted as soon as possible off the column since, although DNA remains intact on the Minicolumn for up to 30 minutes. The eluted plasmid DNA was stored in the microcentrifuge tube at 4.degree. C. or -20.degree. C. (50 .mu.l). Typically, the yield was 0.5-2 .mu.g from a 2 ml culture. For plasmids that underwent additional investigation, the process was scaled up, and they were purified using Quiagen's preparation methods.

Detailed Description Text (276):
An aliquot of Fraction IV containing 500 jig of protein was precipitated by the addition of an equal volume of saturated ammonium sulfate at 4.degree. C., centrifuged at 15,000 rpm at 4.degree. C. in a SS-34 rotor, resuspended in 50 .mu.l Buffer U and dialyzed overnight versus Buffer U at room temperature. The sample was applied to a 5% SDS polyacrylamide tube gel (0.5 cm diameter) and subjected to electrophoresis in an 230A High Performance Electrophoretic Chromatography module (ABS, Inc.). Fractions (25 .mu.l) were collected, aliquots (5 .mu.l), subjected to 7.5% slab SDS-PAGE, and the proteins visualized by silver staining. Fractions containing the highest concentration of 130 kDa protein were pooled, concentrated approximately 30-fold on a Centricon-10 membrane (Amicon) and analyzed by preparative gel electrophoresis on 7.5% SDS-polyacrylamide gels. The gel-fractionated proteins were transferred to a Hyperbond PVDF membrane (Biorad), and the 130 kd protein band excised and subjected to N-terminal amino acid sequence analysis in an 477A Protein Sequencer according to the manufacturer's (ABS, Inc.) instructions. A sequence of 13 amino-acids was obtained: RKLRFAHLHQHTQ (SEQ ID NO: 109). This sequence was aligned with 6/13 residues from the amino terminus M tuberculosis and H. influenzae in a BLAST search, and the alignments are shown in FIG. 11. In this Figure, "M. tuber" refers to M. tuberculosis, while "Tth" refers to T. thermophilus, and "H. infl." refers to "H. influenzae." The numbers in this Figure indicate the amino acid numbers for the respective residues.

Detailed Description Text (307):
For the single-stranded amplification step, the Boehringer Mannheim Expand.TM. PCR system was used and following the manufacturer's recommendations except as noted in the following. Buffer A contained 3.5 Al of 10 mM dNTP mix (Gibco/BRL; 350 .mu.M

final); 0.5 .mu.l of the forward primer (80 .mu.M stock concentration; 400 nM final in 100 .mu.l reaction), 46 .mu.l distilled H.sub.2 0). Buffer B contained 10 .mu.l of 10-fold concentrated Boehringer Mannheim Long Template PCR System Buffer 2 (500 mM Tris-HCl (pH 9.2 at 25.degree. C.), 160 mM ammonium sulfate, 22.5 mM MgCl.sub.2), 37.5 III H.sub.2 O), and 1.5 .mu.l Expand Long Template enzyme mix (mixture of Taq and Pwo DNA Polymerase--5 units total) and 1 .mu.l of T. thermophilus genomic DNA (preparation A, 0.6 .mu.g total). Buffers A and B were combined in a 0.2 ml thin walled tube (Intermountain) and the single primer extension reaction was conducted in an MJ Research thermal cycler as follows.

Detailed Description Text (315):
PacI digested DNA (37 .mu.l containing 3.25 .mu.g DNA) was combined with 12.5 .mu.l 10.times.NEB buffer #3 50 mM Tris HCl, 10 MM MgCl.sub.2, 100 mM NaCl, 1 mM DTT (pH 7.9 at 25.degree. C.), 5 .mu.l PstI (NEB, 25 units), 65.5 .mu.l dH.sub.2 O) and digested for 2 h at 37.degree. C. The digested DNA was precipitated by the addition of 1 .mu.l glycogen, 25 .mu.l sodium acetate and 3 volumes of ethanol, recovered by centrifugation, dissolved in 125 .mu.l dH.sub.2 O, and quantitated with PicoGreen.TM. as described. The concentration was 9 ng/.mu.l (i.e., 1125 ng total recovery). DNA was precipitated again as before and dissolved in 2 .mu.dH.sub.2 O.

Detailed Description Text (385):
The Fr. III pellet is the dissolved in Buffer SP to a concentration of approximately 2 mg/ml, and centrifuged (28,000.times.g, 30 min) to clarify. Fr. III is then be loaded onto a SP Sepharose (Pharmacia; approximately 5 mg protein/ml resin) column equilibrated with buffer SP. After loading, the column is washed with one column volume of Buffer SP, and developed with a 12 column volume gradient of 50 to 600 mM NaCl in Buffer SP at a flow rate of 1 column volume/h; 100 fractions are collected. Chromatography is quantitated and optimized as described for the Q-Sepharose column. Additionally, this column resolves .tau. and .gamma., and conditions are optimized to enable this resolution. In situation where .tau. and .gamma. are not separated by this procedure or the preceding Q Sepharose procedure, hydrophobic chromatography using commercially available resins is conducted. The resulting pooled fractions of .gamma. and .tau. are precipitated with ammonium sulfate as described to Q-Sepharose resulting in Fr. IV.

Detailed Description Text (392):
T. thermophilus SSB is isolated by chromatography of DNA cellulose columns using modifications of the methods of Molineux et al. (Molineux et al., J. Biol. Chem., 249 6090-6098 [1974]). Lysates (Fraction I) are prepared by the methods described in Example 2. Fraction II is prepared by addition of 0.24 g to each ml of Fraction I and precipitates collected as described in Example 2. The collected ammonium sulfate precipitate is dissolved in 20 mM Tris-HCl (pH 7.5), 50 mM NaCl, 10% glycerol, 5 mM .beta.-mercaptoethanol at a concentration of 1 mg protein/ml and applied to a denatured DNA cellulose column equilibrated in the same buffer. A 1.5.times.13 cm column is run for each 100 g of T. thermophilus used as starting material. The column is then washed successively with two column volumes of equilibration buffer at 0.5 column volume/E containing 100, 200, 400, 800, 1600 and 2000 mM NaCl respectively. Fractions eluting from the column are monitored by SDS-PAGE, and the tightest binding fractions that contain proteins between 15,000 Da and 30,000 Da are pooled individually for each protein and subjected to further purification by Q-Sepharose (Pharmacia) chromatography. The pooled fractions are dialyzed against 20 mM Tris-HCl (pH 7.5), 10% glycerol, 5 mM .beta.-mercaptoethanol, and then applied to a Q Sepharose column equilibrated in the same buffer at a load ratio of 2 mg protein/ml resin. The column is eluted with a 20-column gradient from 0-1 M NaCl in the equilibration buffer and the fractions containing the sought protein selected by SDS-PAGE in the preceding step pooled. This procedure is repeated for each major candidate.

Other Reference Publication (73):
Myers and Gelfand, "Reverse transcription and DNA amplification by a Thermus thermophilus DNA polymerase," Biochem., 30:7662-7666 (1991).

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TITLE: Even length proportional amplification of nucleic acids

Drawing Description Text (3):
FIG. 2 depicts an overview of a preferred embodiment of the even length proportional amplification methods of the present invention. Single-stranded cDNA may be produced from total RNA containing a poly(A)+mRNA template in the presence of reverse transcriptase, oligo-dT primer(s), and deoxynucleotide triphosphates (dNTPs) (1). Fragments of the single-stranded cDNA can be created, preferably with the enzymatic compounds of the present invention (2). Terminal transferase may be used to transfer a poly(A) or poly(G) sequence to the 3'-termini of the single-stranded cDNA fragments (3). An oligonucleotide primer, which preferably includes a poly(T) or poly(C) region and a consensus sequence for the T7 RNA polymerase promoter, may be applied to the poly(A) or poly(G) tailed single-stranded cDNA fragments, and second strand DNA synthesis may proceed to yield double-stranded cDNA (4). T4 DNA polymerase may be used preferably to produce blunt ends in the presence of the appropriate dNTPs (5). In vitro transcription of the double-stranded cDNA, preferably with T7 RNA polymerase in the presence of biotinylated, fluorescently labeled, or radiolabeled CTP or UTP, can produce labeled, amplified sense RNA (6).

Drawing Description Text (7):
FIG. 6 depicts an overview of yet another preferred embodiment of the even length proportional amplification methods of the present invention. Single-stranded cDNA may be produced from a poly(A)+mRNA template in the presence of reverse transcriptase, oligo-dT primer(s), and deoxynucleotide triphosphates (dNTPs) (1). Double-stranded DNA may be produced from the single-stranded DNA in the presence of RNaseH, DNA polymerase and DNA ligase (2). Fragments of the double-stranded DNA can be created, preferably with any enzyme capable of cleaving double stranded DNA, or by physical methods such as sonication or shearing (3). Terminal transferase may be used to transfer a poly(A) or poly(G) sequence to the 3'-termini of the double-stranded DNA fragments (4). An oligonucleotide primer, which preferably includes a poly(T) or poly(C) region (and optionally a consensus sequence for the T7 RNA polymerase promoter) (5), may be introduced to the poly(A) or poly(G) tailed double-stranded DNA fragments, and PCR initiated (6). Labeling can occur during PCR by using biotin-labeled primers or by incorporating biotinylated dNTPs. In the alternative, PCR products may be labeled afterwards with biotinylated ddUTP or ddCTP. Where a consensus sequence for the T7 RNA polymerase promoter was incorporated, in vitro transcription may occur to allow additional amplification up to several hundred fold, while facilitating the retention of the relative abundance of each transcript in the original nucleic acid population.

Detailed Description Text (8):
To determine differential gene expression of given cells or tissues, there are many situations in which only a very small amount of cDNA target is available, for example, a single neuron cell and a very small amount of brain tissue. This requires more than million-fold amplifications of the original mRNA population. The present invention addresses the issue of amplifying of DNA or RNA in an unbiased fashion, thus providing the accurate determination of gene expression in even a single cell or a small amount of tissue. One solution to this problem is to randomly digest the cDNA target that is synthesized from mRNA, into equal or roughly equal length fragments, then attach an adapter at both ends of these fragments, and subsequently amplify the fragments by PCR. Random digestion of cDNA target into approximate equal length fragments is the key. Digestion into equal length fragments facilitates unbiased PCR amplification of the original mRNA population. Indeed, the random digestion of DNA or RNA into equal-length fragments can be accomplished by the enzymatic compounds of the present invention.

Detailed Description Text (14):
The single-stranded or double-stranded DNA populations according to the present invention may refer to any mixture of two or more distinct species of single-stranded DNA or double-stranded DNA, which may include DNA representing genomic DNA, genes, gene fragments, oligonucleotides, polypeptides, nucleic acids, PCR products, expressed sequence tags (ESTs), or nucleotide sequences corresponding to known or suspected single nucleotide polymorphisms (SNPs), having nucleotide sequences that may overlap in part or not at all when compared to one another. The species may be distinct based on any chemical or biological differences, including differences in base composition, order, length, or conformation. The single-stranded DNA population may be isolated or produced according to methods known in the art, and may include single-stranded cDNA produced from a mRNA template, single-stranded DNA isolated from double-stranded DNA, or single-stranded DNA synthesized as an oligonucleotide. The double-stranded DNA population may also be isolated according to methods known in the art, such as PCR, reverse transcription, and the like.

Detailed Description Text (15):
Where the nucleic acid sample contains RNA, the RNA may be total RNA, poly(A).sup.+ RNA, mRNA, rRNA, or tRNA, and may be isolated according to methods known in the art. See, e.g . Maniatis, et al., Molecular Cloning: A Laboratory Manual, (Cold Spring Harbor Lab., Cold Spring Harbor, N.Y. 1982) at 188-209. The RNA may be heterogeneous, referring to any mixture of two or more distinct species of RNA. The species may be distinct based on any chemical or biological differences, including differences in base composition, length, or conformation. The RNA may contain full length mRNAs or mRNA fragments (i.e., less than full length) resulting from in vivo, in situ, or in vitro transcriptional events involving corresponding genes, gene fragments, or other DNA templates. In a preferred embodiment, the mRNA population of the present invention may contain single-stranded poly(A)+RNA, which may be obtained from a RNA mixture (e.g., a whole cell RNA preparation), for example, by affinity chromatography purification through an oligo-dT cellulose column.

Detailed Description Text (16):
Where the single-stranded DNA population of the present invention is cDNA produced from a mRNA population, it may be produced according to methods known in the art. See, e.g, Maniatis et al., supra, at 213-46. In a preferred embodiment, a sample population of single-stranded poly(A)+RNA may be used to produce corresponding cDNA in the presence of reverse transcriptase, oligo-dT primer(s) and dNTPs. Reverse transcriptase may be any enzyme that is capable of synthesizing a corresponding cDNA from an RNA template in the presence of the appropriate primers and nucleoside triphosphates. In a preferred embodiment, the reverse transcriptase may be from avian myeloblastosis virus (AMV), Moloney murine leukemia virus (MMuLV) or Rous Sarcoma Virus (RSV), for example, and may be thermal stable enzyme (e.g., hTth DNA polymerase).

Detailed Description Text (19):
In addition, the nucleic acid binding region may comprise non-specific single-stranded DNA binding proteins, e.g., E. coli single-stranded binding protein (SSB). Furthermore, the nucleic acid binding region may comprise a non-specific DNA binding protein that is capable of binding to double-stranded nucleic acids. Such non-specific nucleic acid binding proteins capable of binding double-stranded molecules may include the core histones H2A, H2B, H3, and H4.

Detailed Description Text (20):
In an alternate aspect of the present invention, the nucleic acid binding region may comprise oligonucleotides of specific length that are capable of binding to single-stranded or double-stranded nucleic acids and in a preferred embodiment, comprise a specific footprint of about 30 to 200 nucleotides. One particular embodiment may include producing a set of randomized 30-mers with 1,10-phenanthroline coppers attached at both the 5' and 3' ends of the oligonucleotides. Using the appropriate stringency conditions to facilitate non-specific binding, the randomized 30-mers can bind to both complementary and mismatched sequences on the nucleic acid to be fragmented. The stringency conditions may be optimized for nonspecific binding by changing pH, temperature, time, and salt concentration. See Maniatis, et al.

Detailed Description Text (43):
Fragmentation of Single-Stranded cDNA. Two 1,10-phenanthroline moieties may be

covalently attached, for example, to E. coli SSB protein, by methods known in the art, thus providing a specific footprint on the single-stranded cDNA. 30 .mu.g 1,10-phenanthroline linked SSB protein may be placed in a tube containing 100 ng cDNA, 4 mM Tris-HCL (pH 8.0), 0.45 mM copper sulfate and may be incubated for 30 minutes at room temperature to allow binding of the enzymatic compound to the cDNA. The cleavage reaction is initiated by the addition of mercaptopropionic acid at a final concentration of 4.75 mM. The cleavage reaction may be allowed to proceed for 30 seconds at 20.degree. C. The cleavage reaction may be terminated at the appropriate time by the addition of 2,9-dimethyl-1,10-phenanthroline at a final concentration of 2.3 mM. After termination of the reaction, a phenol/chloroform extraction can be performed, followed by precipitation with 0.1 volumes (v) NaOAc and 2 v ice-cold 100% ethanol. The fragmented cDNA sample may be stored under ethanol at -80.degree. C. overnight or until ready for use. The fragmented cDNA is recovered by centrifugation for 20 minutes at 14,000 g at 4.degree. C. The fragmented cDNA pellet is rinsed with 70% ethanol and allowed to air dry for 5 minutes.

Detailed Description Text (67):
A total RNA population is isolated from tissue or cells and reverse transcribed to produce cDNA. Then, in vitro transcription (IVT) produces biotin-labeled cRNA from the cDNA. The cRNA is fragmented by the method of the present invention before hybridization. Hybridization specificity and sensitivity of longer DNA/RNA targets (>50 bp) with probes on the gene chip is often reduced because of secondary structure and interaction among targets and probes on the array. Even-spacing digestion of DNA/RNA targets into smaller fragments of a roughly equal size eliminates these complications and increase hybridization specificity and sensitivity. In a preferred embodiment, an even-spacing fragmentase with a footprint about 20-30 bp should fragment DNA or RNA target into 20-30 bp in length, which is similar to the length of the probes on the array.

Detailed Description Text (74):
For each probe array to be stained, combine the following components to a total volume of 200 .mu.l (1:100 dilution of SAPE, final concentration of 10 .mu.g/ml): 188 .mu.l 1.times.MES; 10 .mu.l of 50 mg/ml acetylated BSA (final concentration of 2.5 mg/ml); and 2 .mu.l of 1 mg/ml streptavidin phycoerythrin (SAPE).